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10. Thank you!

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ALZHEIMER BETA-PEPTIDE, PROTEIN KINASE C, AND MEMORY.
H. Brockerhoff, V.P.S. Chauhan, H.W. Wisniewski, A. Chauhan. N.Y.S. Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314.

Alzheimer disease (AD) lesions show an accumulation of a "beta-peptide" (BP) with a hydrophilic stretch of 28 and a hydrophobic stretch of 12-16 residues, i.e., with the overall structure of a detergent. Such a peptide may be expected to have fusogenic or membranolytic character, and, at lower than lytic concentration, change the properties of the cellular membranes in which it is embedded; e.g., change enzymic activities. We find that BP acts comparable to the membranolytic protein melittin. An intriguing candidate for further change is protein kinase C, a key phosphorylating enzyme which is reported to be reduced in AD and involved in long-term potentiation, i.e., cellular memory. We find that in vitro exposure of PKC to BP in micellar or liposomal system leads to the inhibition of PKC activity, at micromolar concentration of BP. Since the unrelated peptide, melittin, also inhibits PKC we suspect that a disorganization of the membrane rather than direct PKC-BP bonding causes PKC inhibition. The results suggests the existence of a causal chain from beta-peptide accumulation → inhibition of protein kinase C → cellular memory loss → observable memory loss.

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INVOLVEMENT OF CHOLINERGIC PATHWAYS IN CONTROL OF OXIDATIVE METABOLISM BY RATS EXPOSED TO DIFFERENT ENVIRONMENTAL TEMPERATURES.
S. Krishnan, M. B. Nichols and P. P. Haickel. Dept. of Pharmacol. & Toxicol., Sch. of Pharmacy & Pharmacal Sci., Purdue Univ., W. Lafayette, IN 47907.

Exposure of adult rats to an environmental temperature (ET) of 3-5° C for 24 hrs. slightly increases oxygen consumption (O_2-con) and significantly increases carbon dioxide production (CO_2-pro). After 96 hrs. exposure to the lowered ET, both O_2-con and CO_2-pro are significantly elevated. Exposure of rats to an ET of 31-33° C for 24 or 96 hrs. slightly decreases O_2-con; significant increases in CO_2-pro are seen. A single dose of physostigmine (0.5 mg/kg, s.c.) given to animals maintained at ET of 22-25° C significantly increases both O_2-con and CO_2-pro. In rats exposed to the lowered ET (3-5° C) for 24 hrs., no such effect is seen; after 96 hrs. of exposure, a dramatic increase in O_2-con is evoked by physostigmine. Physostigmine also markedly elevates both O_2-con and CO_2-pro in rats exposed to the elevated ET (31-33° C) for 24 or 96 hrs. Atropine (5.0 mg/kg, s.c.) has minimal effects on O_2-con and CO_2-pro under all ET conditions. In combination with physostigmine, it results in an antagonism, except in rats exposed to the 3-5° C ET for 24 hrs. The results support a role for cholinergic pathways in the control of energy metabolism and may form the basis for a non-invasive procedure for early detection of cholinergic system(s) malfunctions in disease states such as senile dementia.
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AUTO-DESTRUCTION OF CHOLINERGIC NEURONS IN ALZHEIMER'S DISEASE? RAPID AUTOPSY EVIDENCE FOR EXTREME NEURONAL HYPERACTIVITY.
E.J. Seidler, C.B. Nemeroff and T.A. Slotkin. Duke Univ. Med. Ctr., Durham, NC 27710.

Conventional autopsy material demonstrates the loss of cholinergic neurons in specific cerebral cortical areas as one of the hallmarks of Alzheimer's Disease (AD). Using fresh autopsy material (within 2 hr of death), we have evaluated the functioning of cholinergic neurons in patients with confirmed AD and in matched controls. Regions were selected for those most involved in AD (4 cerebral cortical areas), variably involved (hippocampus and caudate) and relatively uninvolved (putamen). For each region, both choline acetyltransferase (ChAT) and synaptosomal high-affinity choline uptake were assayed; ChAT is primarily an index of numbers of nerve terminals (independent of nerve activity), whereas uptake is responsive to activity and rate-limiting in acetylcholine synthesis. Consistent with findings from standard autopsies, we found deficits of ChAT confined to cortical regions in the rapid-autopsy AD population. Nevertheless, choline uptake was increased in these regions. The elevation in uptake, in the face of decreased ChAT (lowered numbers of terminals), resulted in a marked increase in the uptake/ChAT ratio (activity per terminal), suggesting that nerve impulse activity is severely up-regulated in the remaining neurons. This difference became even more significant after values were individually normalized relative to an unaffected region (putamen). These results resemble findings in developing rats, where there is also a period of high intrinsic cortical cholinergic activity; overstimulation, either through nicotine administration or by dietary choline supplementation, leads to neuronal death in this animal model. Because the increase in choline uptake in AD is extreme (an order of magnitude higher than that obtained with convulsants), these results suggest that chronic cholinergic overstimulation could contribute to the death of neurons in AD. (USPHS MH-40524, AG-05128, HD-09713)

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Morphological alterations of neuropeptide systems in the amygdala in Alzheimer's disease (AD).
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It is well recognized that in AD a variety of neurotransmitter and peptide systems are affected, yet to differing extents. Using the amygdala as a model system we sought to determine the similarities and/or differences in the morphology of peptide systems found by biochemical criteria to be either affected (i.e. somatostatin) or unaffected (i.e. substance P and neurotensin) within this nucleus in patients with AD. Histological and acetylcholinesterase histochemical stains were used to define the cytoarchitecture of the amygdala. The topography of the pathologic lesions were determined using Thioflavin-S. Light microscopic examination revealed these three peptide systems to be similarly affected and to be characterized morphologically by gross varicose swellings. These morphologic features were rarely observed within the amygdala of control patients. In AD brains these cytological changes were most prevalent in the areas of the amygdala showing the highest degree of pathology. In many instances the swollen processes were observed within the neuritic portion of the plaque. The similarity in the morphological features between these three peptide systems suggest a common sequence of pathological events which may be undetected by biochemical criteria alone.
This research was supported by NIH grants AG05344 & AG08206.

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A antichymotrypsin-like protein is present in normal human cerebrospinal fluid.
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Cerebrospinal fluid (CSF) from 24 male patients with non-neurologic disease (age 62.5± S.E.M) were analyzed for the presence of an α-1 antichymotrypsin-like protein. A chymotrypsin chromogenic assay (Succinyl-Ala-Ala-Pro-Phe-4PNA) was used to examine the CSF samples. All CSF samples showed inhibitory activity ranging from 45-80 percent inhibition. SDS-PAGE analysis of the samples revealed the presence of a 68 Kd protein migrating identical to authentic human plasma α-1 antichymotrypsin (ACT). Complex formations were preformed with iodinated bovine chymotrypsin of several CSF samples having the highest chymotrypsin-inhibitory activity. Comparison was made with authentic human plasma fibronectin. These studies showed the formation of complexes. α-1 ACT, a serpin, has been detected in amyloid senile plaques in brains of Alzheimer's disease patients. In addition, another serpin, protease nexin I (PNI) also stains these plaques. Recently, the β-amyloid precursor protein (βAPP) has been identified as another serpin, PNII, which is known to form complexes with chymotrypsin as well as the EGF-binding protein.
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A NEW CALPAIN INHIBITOR AND A TOOL TO INVESTIGATE THE CELLULAR BASIS OF SPINAL CORD INJURY.
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Calcium activated neutral proteases (i.e., calpain) are known to promote extensive degradation of cytoskeletal proteins in neurons. The purpose of this study was to develop a unique calpain inhibitor effective in preventing degradation of the neurofilament triplet, a known target of calpain. The spinal cords of Sprague-Dawley rats were isolated and incubated for three hours in one of three solutions: 1) physiological solution with no Ca^{++}; 2) physiological solution with 2mM Ca^{++}; or 3) physiological solution with 2mM Ca^{++} and 0.11 mg of the calpain inhibitor. Subunits of the neurofilament triplet (200 kDa, 160 kDa, 68 kDa) were purified then identified by gradient SDS PAGE. The mean (± SD) neurofilament pellet weight obtained from the spinal cord bathed in the Ca^{++} free medium (i.e., control) was 22.5 ± 6.7 mg. In contrast, cords exposed to the anoxic medium containing 2 mM Ca^{++} showed a substantial loss of the neurofilament pellet weight. The mean (±SD) value was 11.7 ± 3.5 mg which was equivalent to a 48 percent reduction in the pellet weight. The calpain inhibitor proved to be extremely effective in inhibiting calcium activated proteolysis. The mean (±SD) pellet weight was 20.0 ± 6.5 mg, or approximately 89 percent of the control condition. The results of the protein assay performed on the pellets mirrored the finding of the pellet weights. The mean total amount of the protein from the control condition was 4.23 ± 0.50 mg. For the Ca^{++} solution, the mean (±SD) value was 1.97 ± 0.86 mg protein. Finally, for the bathing medium containing the calpain inhibitor, the mean (±SD) value was 3.82 ± 0.31 mg, representing 90 percent of the control value. Scans of the gels revealed that this inhibitor was effective in preventing the loss of each of the three subunits of the neurofilament triplet. This study demonstrates that this inhibitor is an extremely effective in limiting neuronal calcium-activated protein degradation.

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